Induction of the LH Surge in Premenarchal Girls Confirms Early Maturation of the Hypothalamic–Pituitary–Ovarian Axis

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Abstract

Purpose: To determine whether premenarchal girls exhibit positive estradiol feedback similar to regularly cycling adult women when given exogenous estradiol. **Methods:** This was a prospective clinical cohort study at 2 institutions. Nine girls and 6 women received a 7-day course of transdermal estradiol designed to produce physiologic, mid-cycle circulating estradiol levels. Participants collected daily morning urine for luteinizing hormone (LH), estradiol metabolites (E1c), and progesterone metabolites (PdG), corrected for creatinine. Main outcomes were percentage increase in LH from nadir to peak and the absolute value of peak LH between the 2 groups, using *t* testing and linear mixed-effects modeling. **Results:** All participants exhibited a positive feedback response to estradiol. Adult women had a 532.8% (95% confidence interval [CI]: 253.7–1119) increase in LH after estradiol exposure; premenarchal girls had a 497.9% increase (95% CI: 274.5–903.2; *P* = .86). The absolute value of the LH surge in women was 9.50 mLU/mgCr (95% CI: 2.59–43.34.90) and in premenarchal girls was 2.57 mLU/mgCr (95% CI: 0.53–12.49; *P* = .15). **Conclusions:** Premenarchal girls can mount an LH surge proportionally similar to regularly cycling adults. This occurs earlier in puberty than previously believed, in contrast to current dogma that maturation of the hypothalamic–pituitary–ovarian axis occurs after menarche and is the rate-limiting step for the establishment of regular, ovulatory cycles. Failure to achieve regular cycles may instead be due to nutritional or ovarian factors. Young girls who fail to ovulate shortly after menarche may warrant further evaluation for endocrinopathies.

Keywords

estrogen, luteinizing hormone, menstrual cycle

Introduction

The hypothalamic–pituitary–ovarian (HPO) axis is an intricate system composed of positive and negative feedback mechanisms. Prior to puberty, the HPO axis operates at a dampened amplitude of luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion, maintaining gonadal quiescence.¹ Generally, thelarche signals at the start of pubertal development, with menarche debuting 2 to 3 years afterward. Among girls who are well nourished and live in developed countries, the median age at menarche is 12 to 13 years of age, with African American girls experiencing menarche about 5 months earlier than girls of other races.^{2,3} Nutritional status can also influence pubertal development, and girls with more adipose tissue, or a higher body mass index (BMI), begin menstruating earlier. Although not entirely clear, it has been postulated that these patient populations have higher levels of leptin, which may regulate gonadotropin-releasing hormone (GnRH) pulsatility, thereby influencing pubertal onset.⁴

As a female undergoes puberty, a slow, progressive increase in pulsatile GnRH stimulates follicular growth and maturation, leading to estradiol secretion.^{5,6} Mature, adult midcycle levels of estradiol—approximately 200 pg/mL for 50 hours⁷—stimulate the pituitary to release a massive surge of LH, the critical signal for ovulation. A robust LH surge in

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response to a rise in estradiol is needed to establish regular, ovulatory menstrual cycles. This positive estradiol feedback response has long been postulated to be the final step in the maturation of the HPO axis.

Anovulation and longer menstrual cycles are more common shortly after menarche, with about 90% of cycles ranging from 21 to 45 days.⁸ Engle and Shelesnyak studied the menstrual cycles of 100 girls residing in an orphanage in the 1930s by recording their sanitary napkin counts and concluded that cycles trended toward regularity after the first 20 to 24 cycles.⁹ Others have shown that by the third year after menarche, 60% to 80% of cycles are 21 to 34 days, similar to adult women.¹⁰ The clinical corollary of this concept is that, for young women who begin menstruation and do not ovulate monthly, all that is needed is time to complete the process and achieve a consistently ovulatory menstrual cycle pattern. On the basis of these data, many young women with oligomenorrhea are reassured by their health-care providers that irregular menstrual cycles will regulate with time.

There are reasons to be concerned that this clinical concept may no longer be relevant. The population BMI has risen markedly in adolescent girls since the 1930s. Since a critical amount of body fat is considered important in establishing regular menstrual cycles,¹¹ it seems far less likely that girls today have insufficient fat mass exerting restraint on hypothalamic function. Some recent studies have concluded that a positive estradiol feedback response appears in mid-to-late puberty or even prior to menarche.^{12,13} However, Reiter et al¹² studied girls who had reproductive disorders such as Turner syndrome or hypothalamic tumors, and Zhang et al¹³ reported their findings anecdotally and incidentally in 2 research participants. We therefore chose to revisit this question by studying whether or not a brief exposure of the HP axis to estradiol in normal premenarchal girls could result in consistent appearance of an LH surge and at what point in the pubertal transition this maturation might occur. If positive LH feedback is a common, expected phenomenon prior to menarche, then regular, or ovulatory, menses should occur rapidly after the onset of the first menses.

We sought to evaluate the ability of healthy, normal weight, premenarchal girls to exhibit positive estradiol feedback when given an exogenous estradiol challenge designed to mimic the preovulatory rise of estradiol in adult menstrual cycles. Our hypothesis was that a positive feedback response to exogenous estradiol, in the form an LH surge, would be present at least by midpuberty.

Methods

We conducted a prospective clinical cohort study of 9 girls and 6 adult women at 2 separate sites. Girls and women were recruited from the community using web-based advertising, university-wide advertising, and oral communications. The protocol was approved by the institutional review boards at the Albert Einstein College of Medicine and the University of

Colorado School of Medicine. All girls were premenarchal, and none were taking or had ever taken any exogenous reproductive hormones. Inclusion criteria were: (1) age 8 to 15 years, (2) BMI between the 5th and 95th percentile, (3) normal thyroid stimulating hormone (TSH) and prolactin, (4) no excessive exercise (>4 hours per week), and (5) Tanner stage II or III. Girls were staged using the Tanner breast and pubic hair criteria by 1 of 2 physicians (N.S., J.K.) at the time of the initial screening and blood draw.¹ Adult women were recruited as a control group. They were between the ages of 18 and 35 years and had the following inclusion criteria: (1) BMI between 21 and 35 kg/m², (2) regular, monthly cycles every 25 to 35 days with evidence of ovulation (midluteal progesterone >6 ng/mL), (3) no excessive exercise (>4 hours per week), and (4) normal TSH and prolactin. None of the adult women had used any exogenous hormones within the past 3 months. Participants were excluded if they endorsed (1) cigarette smoking, (2) any condition that might affect urinary gonadotropin excretion such a renal disease, (3) evidence of androgen excess at the time of study screening examination (acne or hirsutism), and (4) at the University of Colorado, an additional exclusion was required, because the IRB required that all girls be screened for prothrombotic mutations prior to administering exogenous estradiol.

All adult participants provided informed consent, and parental informed consent and child assent were obtained for all of the girls studied. Each participant received a kit containing ten 12 × 75 mm polypropylene tubes, urine collection cups, storage boxes, written instructions, and a log sheet. After initial screening, participants were mailed 100 µg transdermal estradiol (Climara) patches and were dosed based on weight and body surface area (BSA; <http://medcalc.com/body.html>). Girls with a BSA >1.12 m² were given three 100 µg patches; those with a BSA <1.12 m² were given two 100 µg patches with the goal of achieving a midcycle level of circulating estradiol (150–250 pg/mL). After their first morning void on day 1, each girl was instructed to apply patches to the lower abdomen or back. First morning voided urine was collected each subsequent day for the next 7 days for a total of 8 days of collection. The adult control group was instructed to place patches after their first morning void on day 2 of their menses. The adult control group then followed the same estradiol patch administration and urine collection protocol as did the girls. After first morning urine collection on day 8, both groups were instructed to remove and discard their patches.

Urine was collected into polypropylene tubes with 7% glycerol (a preservative used to maintain gonadotropin immunoactivity)¹⁴ and frozen within 2 hours of collection in each participant's home freezer. Each tube was labeled with the date, the day of collection, and a unique alphanumeric identification number. Participants were instructed to record each collection on the log sheet and to report errors, irregularities, or symptoms. At the end of the week, these specimens were returned to the research center on ice and stored at –20°C until the assays were ready to be run.

Luteinizing hormone (DELFA; Pharmacia, Gaithersburg, Maryland), urinary estrone conjugates (E1c), and pregnanediol-3-glucuronide (PdG) metabolites (in-house ELISA) were measured by urinary assays, which had been previously established and validated.¹⁵ These levels were adjusted for creatinine. The interassay CVs were 5.3% for PdG and 3.8% for E1c. The intra-assay CVs was 2.3% for LH. The LH samples were batched, and each individual participant's samples were run in a single assay; therefore, no inter-assay CV is reportable. Both E1c and PdG were not available for the samples obtained at Albert Einstein College of Medicine, because samples were discarded after LH determinations were performed. Samples from all of the girls recruited at the University of Colorado were assessed for E1c and PdG to confirm compliance and the absence of endogenous progesterone production.

The study was powered based on the assumption that 90% of the adult women would have an LH surge in response to estradiol administration. The null hypothesis was that 20% or fewer of the girls in the Tanner group would have a detectable LH surge; this set of assumptions yielded a sample size of 10 adult women and 8 girls per Tanner group. However, all participants in the study, both adult and child, demonstrated a positive feedback response to estradiol, and therefore, statistical analysis was not possible for this outcome. There was no ability to distinguish LH response by Tanner stage because all girls in both Tanner II and III stages demonstrated an LH surge. This also made further testing of girls in Tanner stages IV and V irrelevant, as we had already identified consistent LH responses at the earlier Tanner stage studied. Therefore, we attempted to examine the nature of the LH response in more detail and assess the percentage change in LH and the absolute amplitude of the LH surge for the adult women and the girls studied. For this analysis, a sample size of 8 per group was estimated to provide 80% power at an α of .05 to detect between-group differences of 25% in LH parameters, assuming a coefficient of variation on the original scale as 0.1, using a 2-sample *t* test.

Data were entered in Excel spreadsheets and reviewed for errors. All participants with complete LH data (at least 1 baseline sample and at least 5 postestradiol samples) were included in the analysis. The study groups were defined as girls and adult women. Luteinizing hormone parameters were defined as peak LH, percentage change from nadir to peak LH. Luteinizing hormone percentage change was calculated for each participant as $(\% \Delta LH) = (LH / \text{Minimum LH}) \times 100$. Differences in LH parameters between groups were the primary outcome. The analysis of LH was on the log scale due to the right-skewed distribution of the hormone, with results reported as geometric mean with 95% confidence interval. Maximum LH was compared between groups using a 2-tailed, 2-sample *t* test. In order to account for correlation from repeated measurements on each participant, $\% \Delta LH$ between groups was compared using a linear mixed-effects model. Within this model, the mean difference between girls versus adult control groups was tested at every day, with time centered at the LH peak. A *P* value < .05 was considered statistically significant.

Table 1. Demographics of Girl Participants.

Participant	Age, years	BMI, kg/m ²	BMI, Percentile	Ethnicity	Breast Tanner Stage	Pubic Tanner Stage
1	11	25.3	95	African American	3	3
2	11	24.3	94	Hispanic	2	3
3	9	18.5	77	Caucasian	2	2
4	11	19.1	70	Hispanic	3	3
5	10	16.6	38	African American	2	1
6	12	18.1	49	Caucasian	3	3
7	10	19.4	81	Hispanic	3	3
8	12	17.2	28	Caucasian	2	2
9	10	22	91	Hispanic	3	2

Abbreviation: BMI, body mass index.

Results

Participants

The study accrued participants from 2 sites in sequence, because the Principal Investigator (N.S.) relocated in the midst of the protocol. All 6 adult women and 5 premenarchal girls were recruited from the Bronx at the Albert Einstein College of Medicine. Following the relocation of the PI (NS) to Colorado, the remaining 4 premenarchal participants were recruited from Aurora, Colorado, and the surrounding Denver Metro area. Table 1 shows the demographics of all the participants and the demographics of the girls alone. Girls ranged in age from 9 to 12 years of age and were of different races, with a BMI range of 16.6 to 25.3 kg/m², which was between the 28th and 95th percentile. All of the girls screened at the University of Colorado were negative for any mutation. Women were between the ages of 19 to 33 years, of different races, and had a BMI 21.5 to 29.1 kg/m². There was 80% compliance with the protocol: 1 premenarchal participant⁶ had 1 patch falloff on day 7 and another⁸ did not collect first morning void on day 3. Data from both of these girls were included in the final analysis.

Luteinizing Hormone Response

The absolute value of the mean peak LH in response to the estradiol challenge in girls was 2.57 mIU/mgCr (95% confidence interval [CI]: 0.53-12.49) and in adult women was 9.50 mIU/mgCr (95% CI: 2.59-34.90). These responses were not statistically significantly different (*P* = .15). The percentage increase in LH during the surge, defined as the percentage change from nadir to peak, was 497.9% in the girls (95% CI: 189 274.5-903.2) and 532.8% in the women (95% CI: 253.7-1119), which were virtually identical (*P* = .86). Centered at LH peak and analyzed over time, both percentage change in LH and absolute LH values demonstrated a similar pattern between girls and women (Figure 1A and B).

Figure 1. Geometric mean and 95% confidence interval for girls and women. A, Absolute value of the luteinizing hormone (LH) surge in girls trended lower than adult women ($P = .15$). B, Premenarchal girls mounted an LH surge similar in amplitude to adult women ($P = .86$).

Estrogen and Progesterone Response to Patch Administration

Urinary E1c confirmed a robust rise in all of the premenarchal girls at the Colorado site, with a range of peak E1c from 45 to 106 ng/mgCr, consistent with adequate E2 exposure. Progesterone metabolites was <2 $\mu\text{g/mgCr}$ in all of the premenarchal girls at the Colorado site, demonstrating the absence of spontaneous ovulation.

Side Effects

Some common side effects of hormonal treatment were reported, including rash under the patch ($n = 1$), mood changes ($n = 1$), breast tenderness ($n = 2$), light spotting ($n = 2$), and mild headache ($n = 1$), but all completed the study. No side effects were reported by the adult women. There were no adverse events.

Discussion

This is, to our knowledge, the first study to examine the characteristics of estradiol-induced LH surges in healthy premenarchal girls at the relatively early Tanner stages II to III. We have demonstrated that premenarchal girls who are otherwise healthy and of normal body weight are able to mount an LH

surge clinically indistinguishable from that of regularly cycling adult women when given exogenous estradiol. These findings challenge the hypothesis that a positive estradiol feedback response is a late pubertal event that may take years after menarche to occur consistently. Our data support the idea that HPO axis maturation occurs relatively early in puberty and would therefore not be expected to lead to irregular or anovulatory menstrual cycles after menarche.

Regular menstrual cycles have been reported to require between 2 and 7 years after menarche to be fully established.^{9,16} Lemarchand et al studied 90 girls and found that by 5 years, only 63% of them exhibited regular, ovulatory cycles.¹⁷ These findings are in contrast with other studies that suggest the positive estradiol feedback response occurs earlier in puberty, well before menarche, and is therefore not the rate-limiting step in the acquisition of regular menstrual cyclicity.^{12,13}

All of the premenarchal girls we studied were able to mount a positive LH response to estradiol, similar to that of regularly cycling ovulatory adult women, as early as Tanner Stage II. Although originally planned, we did not study girls who were Tanner V because our results were uniformly positive prior to that stage. Luteinizing hormone surges were indistinguishable between child and adult in terms of percentage increase. However, the absolute amplitude of the LH surge was less in the children, although not statistically significant. These findings are consistent with the concept that the HP axis is active prior to puberty but operates at an overall suppressed amplitude.^{11,18}

Hypothalamic impairment secondary to marginal nutrition or a low body weight, possibly secondary to the growth spurt that precedes menarche, may preclude a girl from making a robust LH surge. It is known that extreme exercise or anorexia can result in amenorrhea secondary to hypothalamic hypogonadism.¹ In rhesus monkeys, low caloric intake and a lower BMI were associated with anovulatory cycles, while reintroduction of increased calories and a normal BMI led to the return of ovulation.¹⁹ Much of the prior work in humans did not include BMI as a demographic variable and it is thus possible that the girls being studied were nutritionally insufficient. This may well be true of the orphanage cohort reported by Engle and Shelesnyak, as marginal body fat in adolescents has been linked to longer menstrual cycles.^{9,20} Our study selected girls of normal BMI and in good health, and thus, the likelihood that they were nutritionally challenged was negligible.

It is also possible that there are ovarian factors that limit the ability of young women to cycle regularly immediately after experiencing menarche. At the inception of menarche, girls have relatively high levels of circulating anti-Müllerian hormone (AMH). In addition to being a direct marker of ovarian reserve in women, AMH is now known to exert effects on follicle growth and maturation.²¹ Anti-Müllerian hormone has been shown to prevent progression of FSH-induced preantral follicle growth in mouse granulosa cells²² and to inhibit aromatase activity, reduce FSH sensitivity, and reduce estradiol production in human-luteinized granulosa cells.^{23,24} In recently menarchal women, within-woman fluctuations of AMH of up to 54% have been reported to cause reduced

estradiol exposure of the HPO axis, accounting for failure of an appropriate positive feedback signal.²⁵ These effects of AMH may serve to protect the ovarian follicle pool from depletion. At the earliest stages of menstrual cyclicity, it is possible that AMH levels are at or near a threshold, wherein variability in FSH responsiveness may cause intermittent failed folliculogenesis. Therefore, a primary ovarian mechanism may be responsible for oligomenorrhea in the first months after menarche. Thus, the *ovary* and not the hypothalamus or pituitary may be responsible for intermittent early menstrual cyclicity in recently menarchal girls.

There are several strengths and limitations to this study. Strengths include the recruitment of premenarchal girls without any possible confounding medical conditions, which has been a weakness of the prior literature in this area. The study included adult controls against which to measure our outcomes, and the protocol was detailed and involved daily sampling to detect our primary outcome, LH production. The fact that all participants demonstrated a positive LH response to exogenous estradiol is a powerful confirmation that the exogenous estrogen administration was indeed physiologic. On the other hand, there were some weaknesses that limit the interpretation of our findings. Our small sample sizes resulted in the study being underpowered for Tanner group-based outcomes and underpowered to detect a difference in the LH surge amplitude. However, in our original design, we did not anticipate 100% LH surge detection in our sample, and therefore, we chose to examine other aspects of the LH response retrospectively. We also were unable to retain samples from Albert Einstein to confirm E1c and Pdg responses in these participants. However, the similarity of the LH response in all participants implies that the protocol was followed accurately at both sites.

In summary, we conclude that the ability to mount an LH surge in response to preovulatory levels of circulating estradiol occurs early in puberty and prior to menarche. Therefore, failure to achieve regular, ovulatory menstrual cycles may instead be due to nutritional or ovarian factors. Young girls of normal BMI who fail to ovulate shortly after menarche may warrant further evaluation for endocrinopathies before attributing their menstrual irregularity to “HPO axis immaturity.”

Implications and Contributions

The ability to mount an LH surge in response to estradiol occurs early in puberty and prior to menarche. This is in contrast to current belief that maturation of the HPO axis is a late pubertal event, which doesn't occur until after menarche and is thought to be the rate-limiting step for the establishment of regular, ovulatory menstrual cycles. Failure to achieve regular, ovulatory menstrual cycles may instead be due to nutritional or ovarian factors, and therefore, young girls of normal BMI who fail to ovulate shortly after menarche may warrant further evaluation for endocrinopathies.

Declaration of Conflicting Interests

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References

- Hoffman BL, Schorge JO, Bradshaw KD, et al. *Williams Gynecology*. McGraw-Hill Education, 2012. ISBN-13: 978-0071849081.
- Committee Opinion No. 651 Summary: menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Obstet Gynecol*. 2015;126(6):1328.
- Chumlea WC, Schubert CM, Roche AF, et al. Age at menarche and racial comparisons in US girls. *Pediatrics*. 2003;111(1):110-113.
- Hickey M, Balen A. Menstrual disorders in adolescence: investigation and management. *Hum Reprod Update*. 2003;9(5):493-504.
- Lehrer S. Puberty and resonance: a hypothesis. *Mt Sinai J Med*. 1983;50(1):39-43.
- Knobil E. On the control of gonadotropin secretion in the rhesus monkey. *Recent Prog Horm Res*. 1974;30(0):1-46.
- Young JR, Jaffe RB. Strength-duration characteristics of estrogen effects on gonadotropin response to gonadotropin-releasing hormone in women. II. Effects of varying concentrations of estradiol. *J Clin Endocrinol Metab*. 1976;42(3):432-442.
- World Health Organization multicenter study on menstrual and ovulatory patterns in adolescent girls. II. Longitudinal study of menstrual patterns in the early postmenarcheal period, duration of bleeding episodes and menstrual cycles. World Health Organization Task Force on Adolescent Reproductive Health. *J Adolesc Health Care*. 1986;7(4):236-244.
- Engle E, Shelesnyak M. First menstruation and subsequent menstrual cycles of pubertal girls. *Hum Biol*. 1934;6(3):431-453.
- Flug D, Largo RH, Prader A. Menstrual patterns in adolescent Swiss girls: a longitudinal study. *Ann Hum Biol*. 1984;11(6):495-508.
- Grumbach MM. The neuroendocrinology of human puberty revisited. *Horm Res*. 2002;57(suppl 2):2-14.
- Reiter EO, Kulin HE, Hamwood SM. The absence of positive feedback between estrogen and luteinizing hormone in sexually immature girls. *Pediatr Res*. 1974;8(8):740-745.
- Zhang K, Pollack S, Ghods A, et al. Onset of ovulation after menarche in girls: a longitudinal study. *J Clin Endocrinol Metab*. 2008;93(4):1186-1194.
- Santoro N, Goldsmith LT, Heller D, et al. Luteal progesterone relates to histological endometrial maturation in fertile women. *J Clin Endocrinol Metab*. 2000;85(11):4207-4211.
- Santoro N, Brown JR, Adel T, Skurnick JH. Characterization of reproductive hormonal dynamics in the perimenopause. *J Clin Endocrinol Metab*. 1996;81(4):1495-1501.
- Legro RS, Lin HM, Demers LM, Lloyd T. Rapid maturation of the reproductive axis during perimenarche independent of body composition. *J Clin Endocrinol Metab*. 2000;85(3):1021-1025.

17. Lemarchand-Beraud T, Zufferey MM, Reymond M, Rey I. Maturation of the hypothalamo-pituitary-ovarian axis in adolescent girls. *J Clin Endocrinol Metab.* 1982;54(2):241-246.
18. Apter D, Bützow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab.* 1993;76(4):940-949.
19. Lujan ME, Krzemien AA, Reid RL, Van Vugt DA. Developing a model of nutritional amenorrhea in rhesus monkeys. *Endocrinology.* 2006;147(1):483-492.
20. Frisch RE. Body fat, menarche, fitness and fertility. *Hum Reprod.* 1987;2(6):521-533.
21. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertil Steril.* 2011;95(1):170-175.
22. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. *Endocrinology.* 2001;142(11):4891-4899.
23. Chang HM, Klausen C, Leung PC. Antimullerian hormone inhibits follicle-stimulating hormone-induced adenylyl cyclase activation, aromatase expression, and estradiol production in human granulosa-lutein cells. *Fertil Steril.* 2013;100(2):585-592.e1.
24. Grossman MP, Nakajima ST, Fallat ME, Siow Y. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertil Steril.* 2008;89(5 suppl):1364-1370.
25. Hagen CP, Aksglaede L, Sørensen K, et al. Individual serum levels of anti-Mullerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study. *Hum Reprod.* 2012;27(3):325861-325866.